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Original Article

High incidence of acquiring methicillin-resistant *Staphylococcus aureus* in Brazilian children with Atopic Dermatitis and associated risk factors

Eliane D. Abad [a], Dennis de Carvalho Ferreira [b,c,*], Fernanda S. Cavalcante [d], Simone Saintive [a], Ekaterini Goudouris [e], Evandro A. Prado [e], Cristina Hofer [f], Marcia Ribeiro [g], Alexandre Marques Paes da Silva [c], Alexandre S. Rosado [h], Jan Dirk van Elsas [i], Katia R.N. dos Santos [h]

[a] *Pediatric Dermatology Service, IPPMG — Martagão Gesteira Pediatric Institute — Universidade Federal do Rio de Janeiro — UFRJ, Rio de Janeiro, Brazil*
[b] *Veiga de Almeida University, Faculty of Dentistry, Rio de Janeiro, Brazil*
[c] *Estácio de Sá University, Faculty of Dentistry, Rio de Janeiro, Brazil*
[d] *Universidade Federal do Rio de Janeiro — UFRJ, Campus Macaé - Rio de Janeiro, Brazil*
[e] *Pediatric Allergy Service — IPPMG — Martagão Gesteira Pediatric Institute, Universidade Federal do Rio de Janeiro — UFRJ, Rio de Janeiro, Brazil*
[f] *Department of Preventive Medicine, Universidade Federal do Rio de Janeiro — UFRJ, Rio de Janeiro, Brazil*
[g] *Service of Medical Genetics, IPPMG — Martagão Gesteira Pediatric Institute, Universidade Federal do Rio de Janeiro — UFRJ, Rio de Janeiro, Brazil*
[h] *Instituto de Microbiologia Paulo de Góes- Universidade Federal do Rio de Janeiro — UFRJ - Rio de Janeiro, Brazil*
[i] *University of Groningen, Faculty of Mathematics and Natural Science, Microbial Ecology — GELIFES, the Netherlands*

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KEYWORDS
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Abstract *Background:* Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in Atopic Dermatitis (AD) patients can contribute to worsening their clinical condition.

* Corresponding author. Estácio de Sá University, Av. Alfredo Balthazar da Silveira, 580 - Recreio dos Bandeirantes, Rio de Janeiro, RJ, 22790-701, Brazil.
E-mail address: denniscf@gmail.com (D.C. Ferreira).

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S. aureus;
MRSA;
Risk factors

Objective: A cohort study was carried out to determine the incidence of MRSA acquisition and its risk factors in AD children.
Methods: Patients with AD (2 months—14 years old) were followed up for about 1 year at a reference center for AD treatment in Rio de Janeiro, Brazil, from September 2011 to February 2014. Nasal swabs from patients and contacts were collected every 2 months. The SCORAD system assessed the severity of the AD. *S. aureus* isolates were evaluated to determine the methicillin resistance and the clonal lineages.
Results: Among 117 AD patients, 97 (82.9%) were already colonized with *S. aureus* and 26 (22.2%) had MRSA at the first evaluation. The incidence of MRSA acquisition in the cohort study was 27.47% (n = 25). The SCORAD assessments were: mild (46.15%), moderate (37.36%) or severe (16.48%). Risk factors were: colonized MRSA contacts (HR = 2.27; 95% CI: 1.16—7.54), use of cyclosporine (HR = 5.84; 95% CI: 1.70—19.98), moderate or severe AD (HR = 3.26; 95% CI: 1.13—9.37). Protective factors were: availability of running water (HR = 0.21; 95% CI: 0.049—0.96) and use of antihistamines (HR = 0.21; 95% IC: 0.64—0.75). MRSA isolates carried the SCC*mec* type IV and most of them were typed as USA800/ST5.
Conclusions: The high incidence of MRSA acquisition found among AD patients and the risk factors associated show that an effective surveillance of MRSA colonization in these patients is needed.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory disease and one important symptom is the itching that helps spread this skin inflammation. Although present in all age groups, children under five years old are the most affected; the incidence of AD decreases progressively in adulthood.[1] AD is distributed worldwide, although there is a higher prevalence in urban centers. In Brazil, the prevalence of AD is around 6—8% in 6 to 13-year-olds, however among younger individuals this rate is unknown.[2]

In the literature there are several scoring systems for evaluating and monitoring AD: "Severity Scoring Index of Atopic Dermatitis" (SCORAD), "Eczema Area and Severity Index" (EASI), and "Six Area Six Sign Atopic Dermatitis Atopic Score" (SASSAD), among others.[3,4] In addition, the disease can vary from mild to severe, and requires effective therapeutic strategies and support.[5,6]

The etiology of AD has not yet been fully clarified and the current literature describes many factors, including those in the genetic and environmental fields.[7] *Staphylococcus aureus* is an important risk factor for worsening of AD.[8]

Methicillin-resistant *S. aureus* (MRSA) isolates have also been detected in patients with AD and usually carry a staphylococcal chromosome cassette *mec* (SCC*mec*) of community origin, such as types IV or V. Moreover, many studies indicate that the genetic profile of most MRSA isolates recovered from these patients belongs to well-established community lineages.[9,10]

Several studies evaluated the prevalence of *S. aureus* and MRSA colonization in AD patients. Suh et al. observed that *S. aureus* colonization occurred in 80% of AD cases in USA and 16% were MRSA.[11] In New Zealand, 75% of AD pediatric patients were colonized but only 2% with MRSA.[12] In Singapore[13] and in Porto Alegre, Brazil,[14] the authors also observed a high prevalence of *S. aureus* nasal colonization in AD patients, however, MRSA isolates were not found. Also, studies evaluating the incidence of acquiring this pathogen colonization have not been conducted. This study aimed to evaluate the incidence of nasal MRSA acquisition and identify possible risk factors associated with pediatric patients who were being followed-up at an outpatient clinic, through a cohort, of a public university hospital in Rio de Janeiro. In addition, the genotyping of the MRSA isolates recovered from the patients was performed.

Methods

Setting and study design

This prospective cohort study was conducted at the Dermatology and Allergy clinic of a Public University Hospital in Rio de Janeiro Brazil, from September 2011 to February 2014. The study was approved by the Research Ethics Committee under No. 51/11. The age of the patients ranged from two months to 14 years old and all had been diagnosed with AD[15,16] and classified by SCORAD.[3] Patients were included if they and/or their guardians and contacts signed the consent and/or assent form. They were accompanied for one year, with visits every 2—3 months. When a patient missed a visit, a telephone contact was made to reschedule the appointment. At every visit, the patient and contacts answered a questionnaire and nasal swabs were performed.

Exclusion criteria included: patients who had another chronic dermatological disease which compromised the skin barrier (eg. psoriasis and ichthyosis among others); a previous history of MRSA colonization; if MRSA was detected in the nasal swab at the initial visit; if they had any other chronic disease that could increase the risk of MRSA

colonization (eg. cystic fibrosis, acquired immunodeficiency); if they had a history of previous hospitalizations for more than 3 months; dialysis; and if they used percutaneous catheters during the study period.

Demographic and clinical data

Data from questionnaires included: demographic information; if the patient shared a bed with someone; family income; previous use of antibiotics; if any family member is a health care worker; intra-domiciliary contact; had a skin infection at the time of the visit; had any pets. And medication: if the patient used moisturizers and/or a topical cream (corticosteroids, calcineurin inhibitors) and/or oral drugs (immune suppressants - corticosteroids $>$ 1 mg/kg, azathioprine, cyclosporine and antibiotics).

Microbiological characterization

Nasal specimens were cultured on mannitol salt agar (Oxoid, United Kingdom) and characterized by tests standardized by Bannerman and Peacock.[17] All isolates identified as *S. aureus* were subject to the cefoxitin disk diffusion test to detect methicillin resistance.[18] SCC*mec* typing was carried out[19] after extraction of bacterial DNA from the MRSA isolates.[20] Genotyping was performed by pulsed-field gel electrophoresis (PFGE)[21] and multilocus sequence typing (MLST) methods.[22]

Statistical analysis

The data were stored in Microsoft Excel file and analyzed in STATA (StataCorp. 2013. Stata Statistical software. Release 13. College Station TX. StataCorp). Central tendency, dispersion and assessment of distribution were evaluated on all continuous variables. The frequency distribution was described for the categorical variables. In this analysis the dependent variable was the time of the first visit that the patient was MRSA colonized, with censoring at loss of follow up or end of the study. Kaplan–Meier and log-rank tests were used to estimate the time of MRSA acquisition and to evaluate differences among the score categories, respectively. The effect of covariates on the MRSA acquisition was studied using the Cox proportional hazard models for bivariate and multivariate analyses. In the multivariate analysis all independent variables that presented a p-value $<$0.15 in the bivariate analysis were used. Model selection for the multivariate Cox model was based on the likelihood ratio tests.

Logistical problems could make the evaluation of the independent variable MRSA colonization impossible, as it would be necessary to evaluate all the contacts of each child and each child would have a different number of contacts, and most of them were unable to bring all the contacts to the clinic. So, we considered any contact MRSA colonized or not, as an independent variable.

Results

A total of 117 patients with AD were recruited for the study and 97 (82.90%) were already colonized with *S. aureus* at the first visit, where 26 (22.22% of total) patients presented MRSA and 71 (60.68%) presented methicillin-susceptible *S. aureus* (MSSA) isolates. Of the 91 patients followed up in the cohort study (see Fig. 1), 14 (15.38%) dropped out during the study. The average age of the group was 79.67 months/6.6 years old; SD: $\pm$ 4.1 (49.68); ranging from 2.1 to 177.13 months. There was a slight predominance of females (49 patients, 53.85%). The distribution of patients according to the AD severity score was 46.15%, 37.36% and 16.48% for mild, moderate and severe, respectively. The incidence of acquiring MRSA colonization at the end of this cohort was 27.47% (n = 25 patients).

Approximately 28.57% of patients had used antibiotics in the last three months and only 7.69% had had contact with other risk factors such as inpatients or outpatients or relatives working in health care facilities. A total of 41.76% shared a bed for sleeping, 16.48% had pets (dog and/or cat), and 94.51% had running water in their homes (Table 1). Regarding treatments: 89.01% of the patients used steroid cream, 95.60% oral antihistamines, 98.90% moisturizers and 24.17% oral immune suppressants.

The Kaplan–Meier curve analysis showed that a longer follow-up time of these patients increased the risk of MRSA acquisition. When the severity of the disease (SCORAD) and the number of new cases of acquiring MRSA colonization was compared with the follow-up time, 50% of severe cases acquired MRSA colonization within the first 100 days, there is a remarkable difference on MRSA acquisition between the SCORAD mild/moderate group and the SCORAD severe group ($p < 0.001$, log-rank test), as shown in Fig. 2.

A total of 20,618 child/days were followed-up, the median of the follow-up was 210 days, ranging from 60 to 420 days. Some significant data was found in the bivariate analysis: patients had a greater chance of acquiring MRSA colonization when their contacts had skin infections (HR = 7.10; 95% CI: 1.61–31.25). Having running water in the home proved to be a protective effect for MRSA colonization (HR = 0.22; 95% CI: 0.06–0.75) and the use of antihistamines reduced the chances of acquiring MRSA colonization (HR = 0.22; 95% IC: 0.64–0.75). Also, therapeutic interventions and the use of antihistamines seems to serve as a possible protective factor (HR = 0.21; 95% CI: 0.64–0.75), maybe by decreasing the itching. No association was found between the use of ATB and the colonization by the pathogen (HR = 1.60; 95% CI: 0.73–3.92), nor with the variable of sharing the same bed (HR = 1.18; 95% CI: 0.51–2.74).

The multivariate analysis showed that the risk factors independently associated with the MRSA acquisition were: patients who had contacts colonized by MRSA had an increased chance of acquisition (HR = 2.95; 95% CI: 1.16 to 7.54), and the higher the SCORAD the higher the chance of acquiring colonization (HR = 3.26; 95% CI: 1.13–9.37). Also, taking cyclosporine was associated with MRSA acquisition (HR = 5.84; 95% CI: 1.70–19.9). On the other hand, having treated running water in the home decreased the chance of acquiring MRSA colonization (HR = 0.21; 95% CI: 0.04–0.96) (Table 2).

All 24 MRSA isolates identified in the study carried the SCC*mec* IV. Genotypic evaluation was randomly performed on 10 isolates and six of them had profiles related to the USA800/ST5 lineage. One MRSA isolate belonged to the

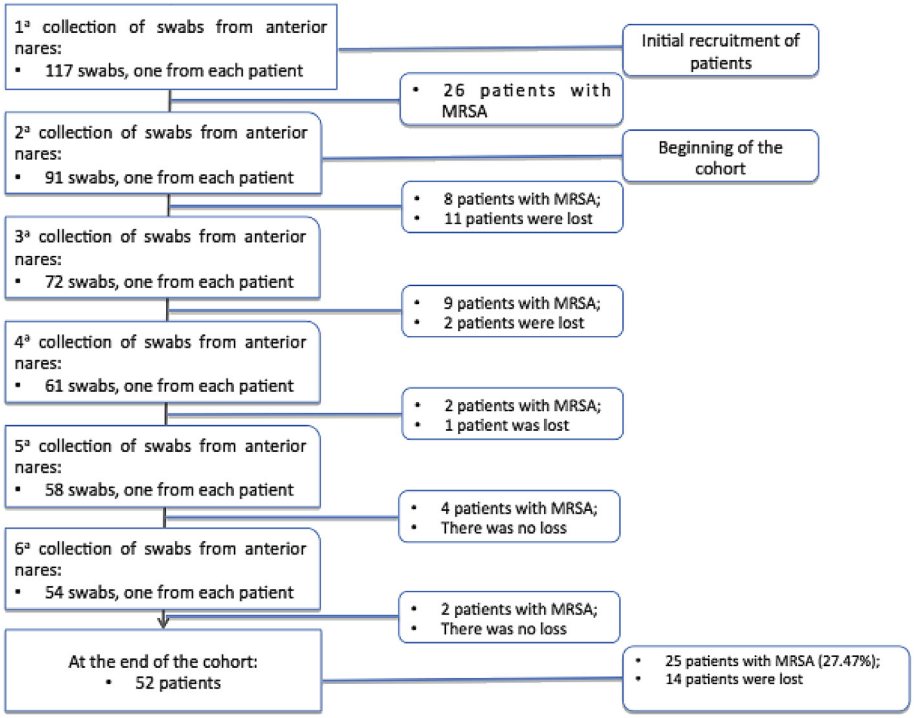

Figure 1. Flowchart of the cohort study and results of MRSA acquisition.

USA1100/ST30 lineage and the other three isolates did not have profiles compatible with any known clonality and were included in STs 1, 45 and 800.

Discussion

Methicillin-resistant *S. aureus* is a major Gram positive pathogen in infectious diseases and it is important in populations where colonization can contribute to the worsening of the clinical picture of a disease, such as in AD patients.[8] To evaluate the incidence of MRSA colonization among AD children is important to determine the risk of acquisition of this pathogen. In this cohort study, after having followed the children for one year a high incidence of nasal MRSA acquisition (27.47%) was detected. In Brazil, Petry and coworkers[14] conducted a two-year clinical follow-up in Porto Alegre to identify AD patients colonized by MRSA isolates. However, they did not find MRSA among the isolates. As far as the authors know, this is the first cohort study that showed the incidence of MRSA acquisition in pediatric patients with AD in Brazil.

In this study, 82.90% of patients were already colonized with *S. aureus* at the first visit and there was a MRSA colonization prevalence of 22.22% (26/117) in patients with AD. In other places like Philadelphia, USA, Suh et al.[11] found 13% of MRSA in AD patients, while in Rome, Italy, Pascolini et al.[23] identified 4.5% (4/117) of atopic children colonized by the pathogen, well below the rates here. In Brazil, although Petry et al.[14] did not detect MRSA in the nasal sites of AD patients they found 68.8% (64/93) of patients colonized by *S. aureus* isolates, lower findings than ours (82.9%) and when compared to values found by other authors.[11,13] The colonization by *S. aureus* in AD patients can contribute to the worsening of the AD infection causing recurrent episodes of skin infections. This phenomenon could be in part due to decreased NMFs ("NMFs - natural moisturizing factor"), reducing the skin humidity and thus altering the skin buffering balance, making it more alkaline. This change increases the action of serine proteases (SE), which increase susceptibility to infection by *S. aureus*.[24]

Moderate and severe AD are related to more skin ailments, a greater area of body affected, more itching and secondary infections.[25,26] In the present study, the diagnosis of moderate or severe AD was associated to a high risk of MRSA acquisition independently compared with mild AD (HR = 3.26; 95% CI 1.13—9.37; p = 0.028). Also, the Kaplan—Meier curve analysis showed that 50% of the patients with severe AD acquired MRSA colonization within the first 100 days ($p < 0.001$). Reports that show MRSA as a risk factor for worsening AD have been based mainly on cross-sectional studies.[27] However, our cohort study also seems to indicate that the disease severity could be a risk factor for MRSA acquisition, reinforcing the relationship between the two. Thus, continuous vigilance is recommended, including a microbiological investigation for patients with AD, especially in patients with moderate or severe AD, since treatment of MRSA infections requires more effective therapeutic strategies with adequate support.

The emergence of MRSA can be linked to the repetitive use of antibiotic therapy, due to itching and to increased cutaneous ailments among other factors.[28] However, in this study the use of antibiotics failed to demonstrate increased risk for acquisition, possibly due to the small sample size, and therefore our data does not corroborate the findings of

Table 1 Bivariate analysis of factors associated with MRSA acquisition in 91 patients with Atopic Dermatitis.

Characteristics	HR	IC 95%	*p*
Attend day care	1.30	0.72—2.40	0.36
Hospitalization in the last six months	0.01	0.00—1.00	0.54
Prior use of ATB	1.60	0.73—3.92	0.21
Contacts with wound and pus at home	**7.10**	**1.61—31.25**	**<0.001**
Animals at home	0.61	0.18—2.06	0.43
Contacts that sleep in the same bed with the child	1.18	0.51—2.74	0.68
Availability of running water	**0.22**	**0.06—0.75**	**0.01**
Use of topical corticosteroids	7.2	0.00—1.00	0.00
Use of topical tacrolimus	1.9	0.87—4.49	0.10
Use of antihistamine	**0.21**	**0.64—0.75**	**0.01**
Use of moisturizer	0.01	0.00—1.00	0.74
Use of corticosteroids > 1 mg	2.7	1.06—6.92	0.03
Use of azathioprine	4.48	0.00—1.00	0.00
Use of cyclosporine	2.18	0.74—6.43	0,15
SCORAD Light versus Moderate + Severe	**1.82**	**1.50—9.76**	**<0.001**
Gender	1.07	0.47—2.43	0.86
Age	1.00	0.99—1.01	0.33
Room per capita	0.41	0.16—1.04	0.06
Income per capita	0.99	0.99—1.00	0.18
Colonized with MRSA at 1st visit	0.72	0.16—3.08	0.66
Colonized at visit	2.25	0.97—5.22	0.05

HR: Hazard Ratio; CI = confidence interval; p = p value.

other authors. On the other hand, the use of systemic antihistamine showed a HR = 0.21 (95% CI 0.64—0.75; p = 0.01), demonstrating its protective factor, as its use reduces scarification and consequent susceptibility to pathogens.[29]

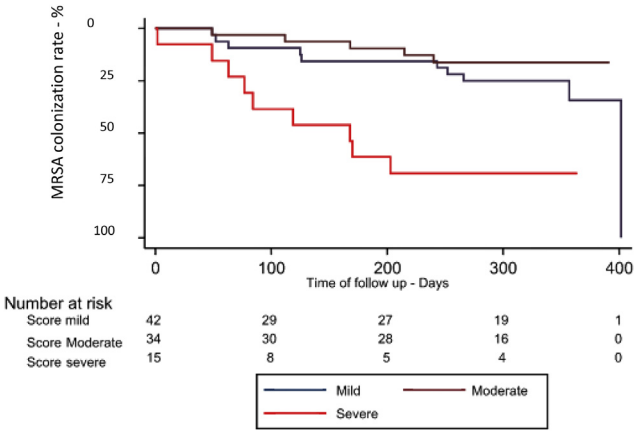

Figure 2. Kaplan—Meier curve: the relationship between MRSA acquisition and SCORAD of patients with Atopic Dermatitis.

Table 2 Multivariate analysis of factors associated with MRSA acquisition in patients with Atopic Dermatitis.

Characteristics	HR	IC	*p*
Contacts colonized with MRSA	2.95	1.16—7.54	0.002
Availability of running water	0.21	0.04—0.96	0.044
Cyclosporine use	5.84	1.70—19.98	0.005
SCORAD:			
Light	0.41	0.13—1.26	0.123
Moderate and Severe	3.26	1.13—9.37	0.028

HR: Hazard Ratio; CI = confidence interval; p = p value.

Cyclosporine is recommended for the treatment of severe AD. This study revealed that its use is associated with the MRSA acquisition independently of any of the other factors analyzed (HR = 5.84; CI 1.70—19.98; p = 0.005). However, Bunikowski et al.[30] observed a reduction of colonization by *S. aureus* in German children with AD using this drug during an eight week follow-up. This study does not mention any MRSA colonized patient and therefore we could suggest that MRSA could have a mechanism to escape the immune system that differs from MSSA. Also, patients in our study could have distinct genetic characteristics that tolerate greater colonization than those investigated by Bunikowski et al.[30]

In the present study the fact that the patient had running water available at home showed a protective effect against acquisition, also independently, reducing the chance of this individual with AD to become colonized by MRSA (HR = 0.21; 95% CI 0.049—0.96; p = 0.044). This finding is in line with the results given by Hennessy et al.[31] in Alaska, where the groups that lived in areas with less access to drinking water had seven times higher risk (RR = 7.1; 95% CI = 3.6—14,0) to have MRSA skin infections. Also the risk of developing skin infections caused by *S. aureus* including MRSA was higher among people who lived in villages with little access to running water than for those with better access to running water.[31]

In the literature there are no reports concerning the relationship of contacts colonized with MRSA being a possible acquisition risk factor for AD patients. In this study, we observed an increased chance, independently, of acquisition of the patient when the contacts were colonized by MRSA (HR = 2.27 CI: 1.16 to 7.54; p = 0.002). In a study carried out in India[32] with 500 children that did not have AD but had skin infections, an association was suggested where contacts who were colonized by MRSA could be a risk factor for these children (p 0.04 95% CI = 1009—2099).

All MRSA isolates identified in this study carried the SCC*mec* type IV. This is the most commonly found type of *mec* cassette among community-acquired MRSA isolates in Brazil.[33] Similarly, Chung et al.[9] also demonstrated the predominance of SCC*mec* IV among MRSA isolates of pediatric patients with AD in South Korea. On the other hand, Lo et al.[10] identified the SCC*mec* V as the prevalent cassette in MRSA isolates from children with AD in Taiwan. Since the SCC*mec* V/ST59 is the most common MRSA lineage present in Taiwan communities, the molecular characteristics of isolates from AD patients seem to be specific to each geographical region. Among 10 MRSA isolates analyzed by

PFGE and MLST in our study, six (60%) belonged to the USA800/ST5 lineage and one (10%) to USA1100/ST30. A recent study conducted by our group with Brazilian children with AD showed that the USA800/ST5 and USA1100/ST30 isolates corresponded to about 60% of the *S. aureus* isolates from patients.[34]

Faced with these multiple aspects involved in *S. aureus* colonization, patients with AD should first be evaluated as to the gravity independent of their status. The clinical evaluation (SCORAD) should be carefully performed, because only when the patient presents an infectious condition and after an evaluation of the bacteria involved should decolonization be considered. Decolonization should not be used as an everyday event, but as a specific event, as there are already reports of mupirocin resistance.[35] Another option such as sodium hypochlorite solution 0.005% has appeared as a good alternative in these cases.[36,37]

The main limitation of this study is that the sample was carried out in an outpatient setting with predominantly mild SCORAD AD patients, which hampered a better comparative analysis for different groups of AD severity. Another aspect that limited the study was the small number of individuals evaluated since there was a loss of 14 patients (15.38%) during follow up, despite efforts to prevent them. This loss was mainly from patients who resided in the greater Rio de Janeiro region and who have difficulties such as access to social mobility.

In conclusion, this study showed an incidence of 27.47% of MRSA acquisition within one year in children and adolescents with AD in Brazil. The main risk factors for MRSA acquisition identified were household contacts colonized and/or infected with MRSA, the use of cyclosporine and the clinical presentations of the disease (AD) as moderate and severe. On the other hand, the use of antihistamine and the availability of running water in the home acted as protective factors decreasing the chance of acquiring MRSA colonization. All MRSA isolates identified in the study carried the SCC*mec* IV and most of the isolates belonged to the USA800/ST5 lineage. These findings call attention to the need for a more effective approach to surveillance of MRSA colonization in order to reduce the number of relapses of the disease and associated infections.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical approval

The present study was approved by the Research Ethics Committee under No. 51/11.

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